D5 reports that TRMo-2 inhibits labelled TSH binding to thyroid membranes: Figure 1(a) indicates about 35% inhibition at 10 μg/ml. It also appears from the data shown in D5 that TRMo-2 also stimulates cAMP production in thyroid membranes (Figure 2(b)). However, these results are suspect because there is no indication as to how the results reported were obtained – for example, for Figure 2 there is no indication as to how the analysis was carried out, nor which labelled ligands were used. For Table V, there is no clear indication as to how the affinities reported were determined. The skilled person is aware that in the absence of the details of experimental protocols, the conclusions drawn in D5 are simply not reliable.

Finally, we note that it is reported in Figure 3 that TRMo-2 was found to react strongly with the TSH receptor on Western blots. However, as noted above the conditions used to run the Western blots in D5 were 2% SDS and 5% mercantoethanol – these conditions are known in the field to stop the TSH receptor binding to TSHR autoantibodies. Therefore, the fact that the D5 antibodies bind to the TSH receptor under these conditions indicates that they are not, in fact, human monoclonal antibodies derived from patient antibodies as defined in claim 1. The SDS-PAGE conditions were such that the TSH receptor must have been denatured. Therefore, the results presented in D5 show that TRMo-2 reacts strongly with denatured TSH receptor. However, this is not a characteristic of patient serum TSH receptor-stimulating autoantibodies: these autoantibodies do not react with the TSH receptor on Western blots (this is an inherent characteristic of such antibodies). Accordingly, the results shown in Figure 3 indicate that whatever the antibodies isolated by Yoshida *et al* in D5, they were not human monoclonal antibodies as defined in claim 1, because such antibodies are not capable of binding to the denatured TSH receptor on Western blots.

In conclusion, therefore, D5 does not provide any convincing evidence that human monoclonal antibodies of any kind have been produced. In particular, there is no evidence that human monoclonal antibodies for the TSH receptor have been produced. The human monoclonal antibodies which D5 purports to have produced have characteristics which are not found in the thyroid-stimulating autoantibodies of Graves' disease patients, therefore these antibodies cannot be monoclonal antibodies corresponding to patient autoantibodies. Therefore, D5 does not disclose human monoclonal antibodies as defined in claim 1, and claim 1 (and all remaining claims, which include the features of claim 1) are novel over D5

Regarding claim 15 in particular, which is directed to an antibody defined structurally, in terms of sequence, D5 does not disclose any antibodies having the claimed sequence and therefore claim 15 and its dependent claims are novel over D5.

5.2 Valente *et al* (D6)

D6 reports a series of molecules which are described as "human monoclonal antibodies to the TSH receptor". However, the skilled person would not consider D6 to be a convincing disclosure of such antibodies for the following reasons.

Firstly, the methods used to isolate the "monoclonal antibodies" are not described in sufficient detail to allow the skilled person to reasonably expect that human monoclonal antibodies would result. D6 reports that six patients with "active Graves' disease" were used. However, no details of these patients are given and in particular no details of serum TSH receptor autoantibody levels are described. Lymphocytes were isolated from the peripheral blood of the six patients, fused with mouse myeloma cells and cultured. Cultures were screened for the presence of antibodies to the TSH receptor using an assay based on

interaction of culture supernatants with bovine thyroid membranes followed by detection of any bound antibody using ^{125}I-labelled protein A or anti-human IgG. Positives were then screened again with ^{125}I-labelled protein A or anti-human IgG in the presence of TSH, such that TSH should block the binding of the labelled protein A or anti-human IgG to any antibodies to the TSH receptor: samples that reacted with the thyroid membranes in the first screening but which were blocked by unlabelled TSH in the second screening were considered to be TSH receptor autoantibodies. However, and as would be apparent to the skilled person, this screening procedure is not specific: it will detect any protein A-reactive material (or any human IgG), and will not detect only TSH receptor antibodies. The second stage of the screening procedure (blocking with unlabelled TSH) is unlikely to provide any specificity because TSH receptor autoantibody binding to the TSH receptor is of such a high affinity that it is not readily inhibited by TSH.

After the screening steps, D6 reports that culture supernatants were purified by protein A affinity chromatography or ammonium sulphate precipitation. Again, these purification procedures are not specific for antibodies to the TSH receptor. Analysis of protein A purified fractions revealed homogenous immunoglobulin preparations with a heavy chain of 50,000 Daltons, which is consistent with IgG (but, of course, gives no indication of antibody specificity). We also note that there is no mention of the light chain of the purified antibodies. The screening and purification steps used in D6 are therefore not sufficient to ensure that monoclonal antibodies with the desired specificity are obtained.

There is also no mention in D6 of expression levels or clone stability. Furthermore, there is no clear evidence in D6 that (a) stable single clones of hybrid cells have been produced or (b) the cultures produce actual human monoclonal antibodies (of any specificity).

Secondly, the assays used in D6 to characterise the "monoclonal antibodies" obtained are inadequate to characterise the antibodies. The results of the assays do not indicate that the "monoclonal antibodies" isolated in D6 are human monoclonal antibodies as defined in claim 1.

In particular, the assays used in D6 include the following:

(i) inhibition of ^{125}I-labelled TSH binding;
(ii) stimulation of cAMP production (or thymidine uptake) in a rat thyroid cell line (FRTL cells) and human thyroid cells;
(iii) stimulation of release of ^{125}I from mouse thyroids in vivo (McKenzie assay); and
(iv) inhibition of ^{125}I-labelled TSH binding to various components of thyroid membranes, in particular gangliosides and glycoprotein components in lysosomes.

D6 reports that four "monoclonal antibodies" inhibit TSH binding to human thyroid membranes (see Figure 1). Two of these (208F7 and 206H3) are also reported to stimulate cAMP production. However, in addition to these activities, these two molecules are also taught to interact with human thyroid gangliosides. Patient serum autoantibodies do not interact with human thyroid gangliosides – rather, they bind to amino acids in the extra-cellular domain of the TSH receptor. We note that the antibodies of the invention also bind

to amino acids in the TSHR extracellular domain (e.g. hMAb TSHR1 binds particularly to amino acids 22 to 260 of the TSH receptor) therefore unlike the D6 antibodies, the antibodies of the present invention have the characteristics of patient serum autoantibodies in the way they bind to the TSH receptor. The D6 antibodies, however, do not have this characteristic.

The D6 antibodies 208F7 and 206Hs are also reported to have a *short* course of action in stimulating the TSH receptor (see Figure 3A). This is in distinct contrast to patient serum autoantibodies to the TSH receptor, which are well known to have *long* acting thyroid-stimulating effects (in this regard we enclose a 2009 paper by Rees Smith *et al*: Horm. Metab. Res. 2009; 41: 448 – 455). Therefore, it is clear that in this respect also, the D6 antibodies do not have the characteristics of patient serum TSH receptor autoantibodies with respect to stimulation of cAMP production by the TSH receptor, as required by claim 1.

In summary, therefore, Valente *et al* have not provided convincing evidence that human monoclonal antibodies of any specificity have been produced, still less that human monoclonal antibodies for the TSH receptor and having the functional characteristics set out in claim 1 have been produced. In addition, the human monoclonal antibodies Valente *et al* claim to have produced have characteristics which are different from the thyroid stimulating autoantibodies found in the serum of patients with Graves' disease – this supports our contention that these antibodies are not what Valente *et al* report them to be. In view of the above comments, it is clear that the antibodies disclosed in D6 are not novelty destroying for the present claims: claim 1 is novel over this document.

In addition, and as for D5 above, we also note that D6 does not disclose any antibodies having the structural features in terms of sequence defined in claim 15. Accordingly, claim 15 is also novel over D6.

As discussed above, it is clear to the skilled person on reading either D5 or D6 alone (even without the later-published documents previously submitted to the examiner which throw significant doubts on the claims made in these papers) that these two articles do not contain any proof that human monoclonal antibodies to the TSH receptor had been obtained prior to the present invention, because of the omissions and contradictions within these articles. The present claims are therefore novel over both D5 and D6.